

# Rediscovery of *Lycodon gammiei* (Blanford, 1878) (Serpentes, Colubridae) in Xizang, China, with comments on its systematic position

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## Abstract

*Lycodon gammiei* (Blanford, 1878), a rarely encountered species of Asian snake, is characterized by ambiguous systematics and biology. Based on a sole specimen of *L. gammiei* rediscovered in southeastern Xizang, China, we conduct a detailed morphological examination and description, and investigate the systematic position of this species. Morphologically, the newly collected specimen is closely aligned with specimens previously described. Mitochondrial DNA-based phylogenetic analyses reveal that *L. gammiei* constitutes an independent evolutionary lineage, forming a clade with *L. fasciatus* (Anderson, 1879), *L. gongshan* Vogel & Luo, 2011, *L. butleri* Boulenger, 1900, and *L. cavernicolus* Grismer, Quah, Anuar, Muin, Wood & Nor, 2014. The closest genetic distance between *L. gammiei* and its congeners was 10.2%. The discovery of *L. gammiei* in Medog County, China, signifies an eastward expansion of its known geographical distribution.

**Key words:** Himalayas, phylogeny, Qinghai-Xizang Plateau, snake



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## Introduction

Exploring the boundaries of geographic distribution and systematic position of species is crucial for understanding their evolutionary origins and diversification and for devising appropriate conservation strategies. Despite considerable progress in recent years, many species remain poorly known and explored. This is particularly evident for some snake species due to their rarity and cryptic habitats.

*Lycodon gammiei* (Blanford, 1878), a rare non-venomous snake species within the family Colubridae, was initially described as *Ophites gammiei* based on a single specimen collected from Darjeeling, West Bengal, India (Blanford 1878). Subsequently, it was reclassified into the genus *Lycodon* (Boulenger 1890) or *Dinodon* (Wall 1923; Smith 1943), identifying it as *Lycodon gammiei*. Wall (1911) compared *L. gammiei* and *L. fasciatus* (Anderson, 1879), and he synonymized *L. fasciatus* with *L. gammiei*. However, Wall (1923) later revised this view,

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recognizing its distinctiveness and validity of *L. fasciatus*. Mahendra (1984) proposed that *L. gammiei* was a color variety of *L. septentrionalis* (Gunther, 1875), while this synonymy was not accepted by all authors. Since its initial description, *L. gammiei* has been found in southeastern Xizang, China (Agarwal et al. 2010) and in Bhutan (Wangyal 2013). To date, however, few specimens of the species have been collected, and no genetic data have been reported.

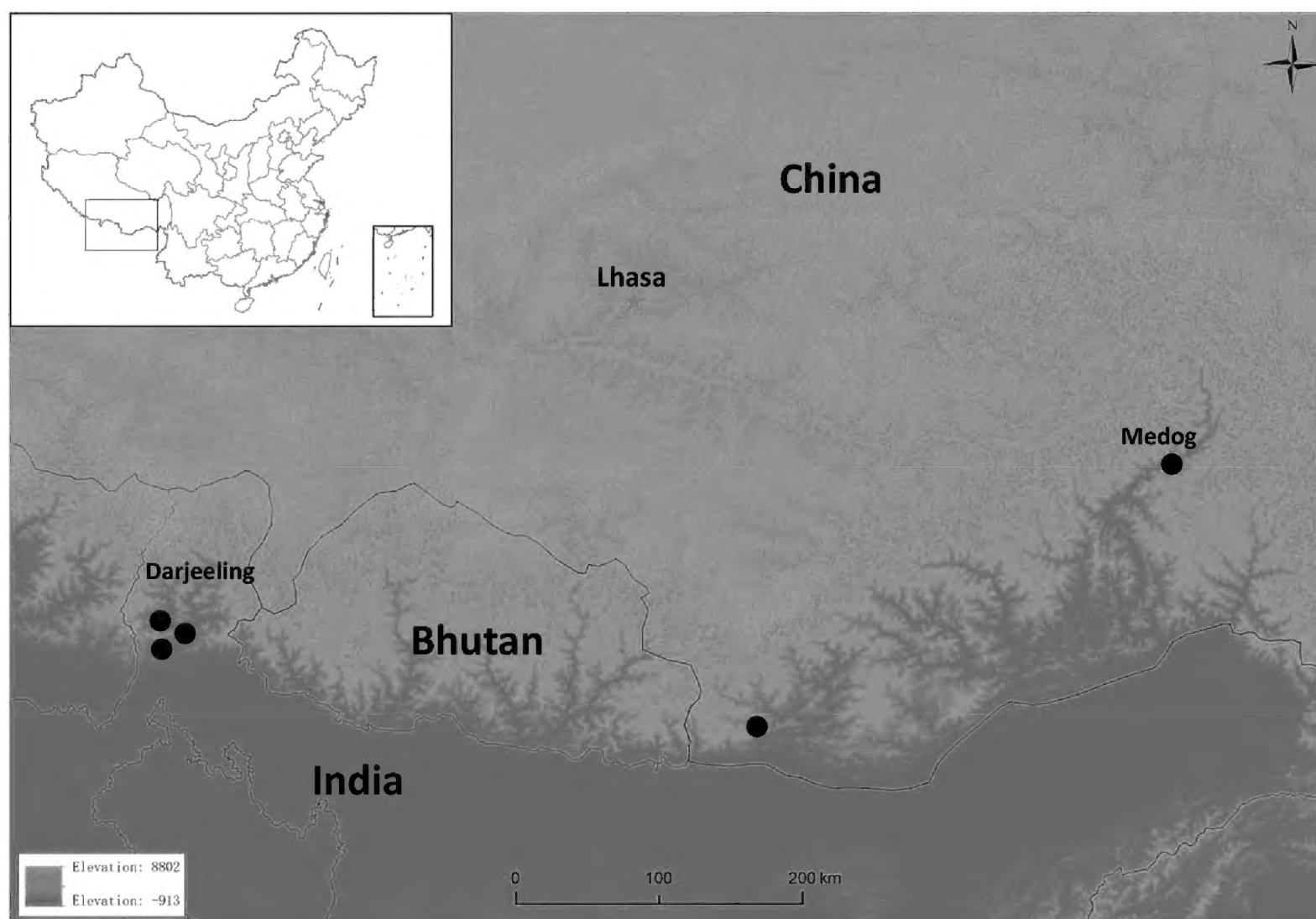
In 2023, we collected a living specimen of *L. gammiei* in Medog County, southeastern Xizang, China. The rediscovery of this species in Xizang not only extends this species' geographic distribution but also allows the exploration of its systematic position through molecular data.

## Materials and methods

### Morphological examination

The specimen deposited at Yibin University (YBU230088) was collected in Beibeng Town, Medog County, southeastern Xizang, China (29°14'02"N, 95°10'38"E) (Fig. 1) on 14 August 2023 at an elevation of 1,431 m by Xiaoqi Mi. The snake was found on a tree near a road at 23:30 hours. Characters relating to scalation, color pattern, and body proportions were recorded from the preserved specimen in laboratory. Snout–vent length (SVL) and tail length (TL) were measured using a meter ruler to the nearest 0.5 centimeter, while all remaining measurements were taken using digital calipers to the nearest millimeter. Symmetric mensural head characters were taken on the right side unless unavailable (e.g. damaged), while meristic characters were recorded on both sides and reported in left/right order.

Comparative data of other specimens of this species were taken from the literature (Blanford 1878; Mistry et al. 2007; Chettri and Bhupathy 2009; Wangyal 2013).



**Figure 1.** Map showing currently known localities of *Lycodon gammiei*.

Molecular phylogeny

Genomic DNA was extracted from the liver tissue of the newly collected specimen using an Animal Genomic DNA Purification Kit (TIANGEN Bio-tech Co., Ltd, Beijing, China). Subsequently, a fragment of the mitochondrial gene cytochrome b (cyt b) was amplified using primers H14919 (5'-AACCACCGTTGT-TATTCAACT-3') and L16064 (5'-CTTTGGTTTACAAGAACAATGCTTTA-3') (Burbrink et al. 2000). The polymerase chain reaction (PCR) products were purified and sequenced in both directions by Sangon Biotech Co., Ltd (Chengdu, China). The obtained sequences were manually edited using SeqMan in Lasergene v. 7.1 (DNASTAR, USA), and aligned using the ClustalW algorithm with default parameters in MEGA v. 7.0 (Kumar et al. 2016), followed by a visual inspection for minor manual adjustments. The DNA sequences were translated into amino acid sequences using MEGA v. 7.0 to verify sequence quality and detect any unexpected stop codons (Kumar et al. 2016). Furthermore, 80 additional sequences were downloaded from GenBank (Table 1).

Both Bayesian-inference (BI) and maximum-likelihood (ML) analyses were executed for the final dataset. Prior to analyses, the best-fit model of nucleotide substitution was selected for each partition (codon position) using Akaike In-

Table 1. Detail information for the samples used in this study.

No.	Species	Voucher Number	Locality	GenBank No.
1	<i>Lycodon albofuscus</i>	LSUHC 3867	–	KX660500
2	<i>Lycodon albofuscus</i>	LSUHC 4588	–	KX660501
3	<i>Lycodon alcalai</i>	KU 327847	Barangay San Antonio, Batanes Province, Philippines	KC010344
4	<i>Lycodon alcalai</i>	KU 327848	Municipality of Sabtang, Batanes, Philippines	KC010345
5	<i>Lycodon anakradaya</i>	SIEZC 20247	Song Giang River, Khanh Hoa Province, Vietnam	OM674283
6	<i>Lycodon anakradaya</i>	SIEZC 20248	Song Giang River, Khanh Hoa Province, Vietnam	OM674284
7	<i>Lycodon aulicus</i>	KU 315378	Tablas Island, Romblon Province, Philippines	KC010350
8	<i>Lycodon aulicus</i>	PNM 7705	Leyte Island, Leyte province, Philippines	KC010349
9	<i>Lycodon banksi</i>	VNUF R2015.20	Khammouane, Laos	MH669272
10	<i>Lycodon bibonius</i>	KU 304589	Cagayan, Philippines	KC010351
11	<i>Lycodon butleri</i>	LSUHC 8365	Perak, Malaysia	KJ607892
12	<i>Lycodon butleri</i>	LSUHC 9137	Perak, Malaysia	KJ607891
13	<i>Lycodon capucinus</i>	–	–	MK844525
14	<i>Lycodon capucinus</i>	MVZ 291703	Timor	MK844522
15	<i>Lycodon capucinus</i>	MVZ 291704	Timor	MK844523
16	<i>Lycodon cathaya</i>	SYS r001542	Longsheng County, Guangxi, China	MT602075
17	<i>Lycodon cathaya</i>	SYS r001630	Longsheng County, Guangxi, China	MT602076
18	<i>Lycodon cavernicolus</i>	LSUHC 10500	Perlis, Malaysia	KJ607890
19	<i>Lycodon cavernicolus</i>	LSUHC 9985	Perlis, Malaysia	KJ607889
20	<i>Lycodon cf. flavozonatus</i>	KIZ 032400	Zayu, Xizang, China	MW199792
21	<i>Lycodon chapaensis</i>	KIZ 27593	Tengchong, Yunnan, China	MW353741
22	<i>Lycodon chapaensis</i>	KIZ 35013	Lushui, Yunnan, China	MW353742
23	<i>Lycodon chrysoprateros</i>	KU 307720	Cagayan, Philippines	KC010360
24	<i>Lycodon deccanensis</i>	–	Tumkur District, Karnataka, India	MW006487
25	<i>Lycodon deccanensis</i>	NCBS NRC AA0010	Karnataka, India	MW006486
26	<i>Lycodon dumerilii</i>	KU 305168	Dinagat Island, Philippines	KC010362
27	<i>Lycodon dumerilii</i>	KU 319989	Mindanao Island, Agusan del Sur Province, Philippines	KC010361
28	<i>Lycodon dumerilii</i>	PNM 7751	Leyte Island, Leyte Province, Philippines	KC010363



No.	Species	Voucher Number	Locality	GenBank No.
29	<i>Lycodon effraenis</i>	KU 328526	Karome, Nakhon Si Thammarat, Thailand	KC010364
30	<i>Lycodon effraenis</i>	LSUHC 9670	Kedah, West Malaysia	KC010376
31	<i>Lycodon fasciatus</i>	CHS 837	Yunnan, China	MK201559
32	<i>Lycodon fasciatus</i>	KIZ 46120	Himalayan region in China	MW111468
33	<i>Lycodon flavicollis</i>	–	Devarayanadurga, Karnataka, India	MW006488
34	<i>Lycodon flavicollis</i>	AIWC 081	India	MZ029434
35	<i>Lycodon flavozonatus</i>	KIZ 023279	Xizang, China	MW199789
36	<i>Lycodon flavozonatus</i>	KIZ 07067	Xizang, China	MW199790
37	<i>Lycodon futsingensis</i>	CHS 670	Nankunshan, Guangdong, China	MK201463
38	<i>Lycodon futsingensis</i>	CHS 751	Guangdong, China	MK201504
39	<i>Lycodon gammiei</i>	YBU 230088	Medog, Xizang, China	<b>OR842906</b>
40	<i>Lycodon gongshan</i>	GP 3547	Lingcang, Yunnan, China	KP901025
41	<i>Lycodon gongshan</i>	GP 3548	Lingcang, Yunnan, China	KP901026
42	<i>Lycodon jara</i>	CAS 235387	Putao, Kachin, Myanmar	KC010367
43	<i>Lycodon laoensis</i>	FMNH 258659	Salavan, Laos	KC010368
44	<i>Lycodon laoensis</i>	LSUHC 8481	O’Lakmeas, Pursat Province, Cambodia	KC010370
45	<i>Lycodon liuchengchaoi</i>	CHS 158	Sanjiazhai, Yunnan, China	MK201303
46	<i>Lycodon liuchengchaoi</i>	CHS 843	Ningshan, Shaanxi, China	MK201563
47	<i>Lycodon liuchengchaoi</i>	CHS 873	Shennongjia, Hubei, China	MK201580
48	<i>Lycodon mackinnoni</i>	ADR 197	Dhobighat, BWLS, Mussoorie, Uttarakhand	MW862977
49	<i>Lycodon meridionalis</i>	CHS 870	Hechi, Guangxi, China	MK201578
50	<i>Lycodon meridionalis</i>	VNUF R2012.4	Bac Kan, Vietnam	MH669271
51	<i>Lycodon meridionalis</i>	VNUF R2017.123	Thanh Hoa Province, Vietnam	MH669270
52	<i>Lycodon muelleri</i>	DLSUD 031	Luzon Island, Cavite Province, Philippines	KC010373
53	<i>Lycodon muelleri</i>	KU 313891	Luzon Island, Camarines Norte Province, Philippines	KC010375
54	<i>Lycodon muelleri</i>	KU 323384	Luzon Island, Aurora Province, Philippines	KC010374
55	<i>Lycodon namdongensis</i>	VNUF R2017.23	ThanhHoa, Vietnam	MK585007
56	<i>Lycodon obvelatus</i>	KIZ 040146	Panzhihua, Sichuan, China	MW353745
57	<i>Lycodon pictus</i>	CIB 115609	Longzhou, Guangxi, China	MT845095
58	<i>Lycodon pictus</i>	VNMN 011227	Ha Lang, Cao Bang, Vietnam,	MT845094
59	<i>Lycodon rosozonatus</i>	CHS 794	Jianfengling, Hainan, China	MK201531
60	<i>Lycodon rufozonatus</i>	CHS 601	Huangshan, Anhui, China	MK201427
61	<i>Lycodon rufozonatus</i>	CHS 710	Yingpanxu, Hunan, China	MK201482
62	<i>Lycodon ruhstrati</i>	CHS 776	Guangxi, China	MK201521
63	<i>Lycodon ruhstrati</i>	CHS 803	Huaping, Guangxi, China	MK201538
64	<i>Lycodon semicarinatus</i>	KUZJPN 28044	–	LC640371
65	<i>Lycodon septentrionalis</i>	CHS 162	Yunnan, China	MK201305
66	<i>Lycodon septentrionalis</i>	KIZ 46117	Xizang, China	MW199801
67	<i>Lycodon serratus</i>	KIZ 038335	Deqin, Yunnan, China	MW353746
68	<i>Lycodon stormi</i>	JAM 7487	Air Terjun Moramo, Sulawesi, Indonesia	KC010380
69	<i>Lycodon striatus</i>	–	Savandurga, Karnataka, India	MW006489
70	<i>Lycodon striatus</i>	CUHC 10368	Pakistan	OQ282988
71	<i>Lycodon striatus</i>	CUHC 11257	–	OQ282989
72	<i>Lycodon striatus</i>	CUHC 9457	–	OQ282987
73	<i>Lycodon subcinctus</i>	CHS 734	Guangdong, China	MK201493
74	<i>Lycodon subcinctus</i>	CHS 797	Diaoluoshan Mountain, Hainan, China	MK201534
75	<i>Lycodon synaptor</i>	GP 3515	Lingcang, Yunnan, China	KP901021
76	<i>Lycodon synaptor</i>	KIZ 046953	Xizang, China	MW199805
77	<i>Lycodon truongi</i>	SIEZC 20249	Song Giang River, Khanh Hoa Province, Vietnam	OM674282
78	<i>Lycodon zawi</i>	CAS 210323	Thabakesay, Saging, Myanmar	AF471040
79	<i>Lycodon zawi</i>	CAS 239944	Kyaukpyu, RakhineState, Myanmar	KC010386
80	<i>Lycodon zayuensis</i>	GP 7327	Zayu, Xizang, China	OP434398
81	<i>Lycodon zayuensis</i>	GP 7329	Zayu, Xizang, China	OP434399

formation Criterion (AIC) implemented in PartitionFinder (Lanfear et al. 2012). The BI analyses were conducted using MrBayes v. 3.2.2 (Ronquist et al. 2012). Searches consisted of three independent runs, each involving four Markov chains (three heated chains and one cold chain), with 10 million generations, sampling every 2,000 generations and with 25% of initial samples discarded as burn-in. Convergence was determined via effective sample size ( $ESS > 200$ ) and likelihood plots against time using Tracer v. 1.7 (Rambaut et al. 2018). The resulting trees were combined to determine the posterior probabilities (PP) for each node based on a 50% majority-rule consensus tree. The ML trees were constructed in IQ-tree (Lam-Tung et al. 2015) using the GTRCAT model and the same partitioning scheme. In total, 1,000 Ultrafast bootstraps (UFB) topological replicates were performed for branch support assessment. *Boiga cynodon* (Boie, 1827) was selected as the outgroup following previous research (Guo et al. 2013).

Uncorrected genetic distance ( $p$ -distance) was calculated in MEGA v. 7.0 (Kumar et al. 2016).

## Results

### Morphological description

Female, SVL 698 mm and TL 223 mm. Body elongated; head rather flattened; snout blunt. Rostral large, trapezoid; internasals much broader than long; prefrontals 3.0 mm in length, distinctly wider than long, extending beyond both sides and touching preocular and loreal; frontal peltate, 4.6 mm in length and 4.1 mm in width; parietals subrectangular, 7.9 mm in length and 4.2 mm in width. Nasals large, nostril located anteriorly and opening backward; loreal scale 1, long, nearly rectangular, failing to touch eye; preocular 1, postoculars 2; temporals 2+2+3. Supralabials 8, 1<sup>st</sup> small, 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> entering orbit, 6<sup>th</sup> highest, 7<sup>th</sup> largest; infralabials 10, first pair in contact, 1<sup>st</sup> to 5<sup>th</sup> in contact with anterior chin shields. Chin shield pairs 2, elongate, anterior pair slightly larger than latter pair. Dorsal scales 17-17-15 rows, scales weakly keeled, except for outermost several rows; scales reduced from 17 to 15 at 143<sup>rd</sup> ventral position. Ventrals 228 (+ 1 preventral); cloacal plate entire; subcaudals 106, paired, dorsal scales of the tail reduced from 6 to 4 at 16<sup>th</sup> subcaudal position.

Head black, with yellow spots or short lines on some shields. Large, yellow spots on each side of posterior part of head. Conspicuous yellow collar on neck. Supralabials and anterior infralabials light yellow with dusky margins. Body surrounded by alternating dusky and light-yellow rings with very irregular, crooked margins. Yellow rings on body totaling 43, first pale ring clear above, anterior dark patch not continuous across throat, remaining rings encircling body. Lower part of head and neck light yellow. On belly, across anterior part of body, dark rings only about half as broad as light-yellow rings, less difference above, dark rings near head much broader above than white rings. Yellow rings on tail totaling 21 (Fig. 2). Preserved specimen somewhat faded, with no yellow visible (Fig. 3).

### Molecular phylogeny

In total, 1,047 bp of sequence data from 84 samples were aligned, with the generated novel sequence deposited in GenBank (Table 1). No deletions, insertions, or stop codons were detected, indicating that unintentional amplification of



**Figure 2.** General view of the studied specimen (YBU 230088) in life and its microhabitat a big tree trunk (by XQ Mi).

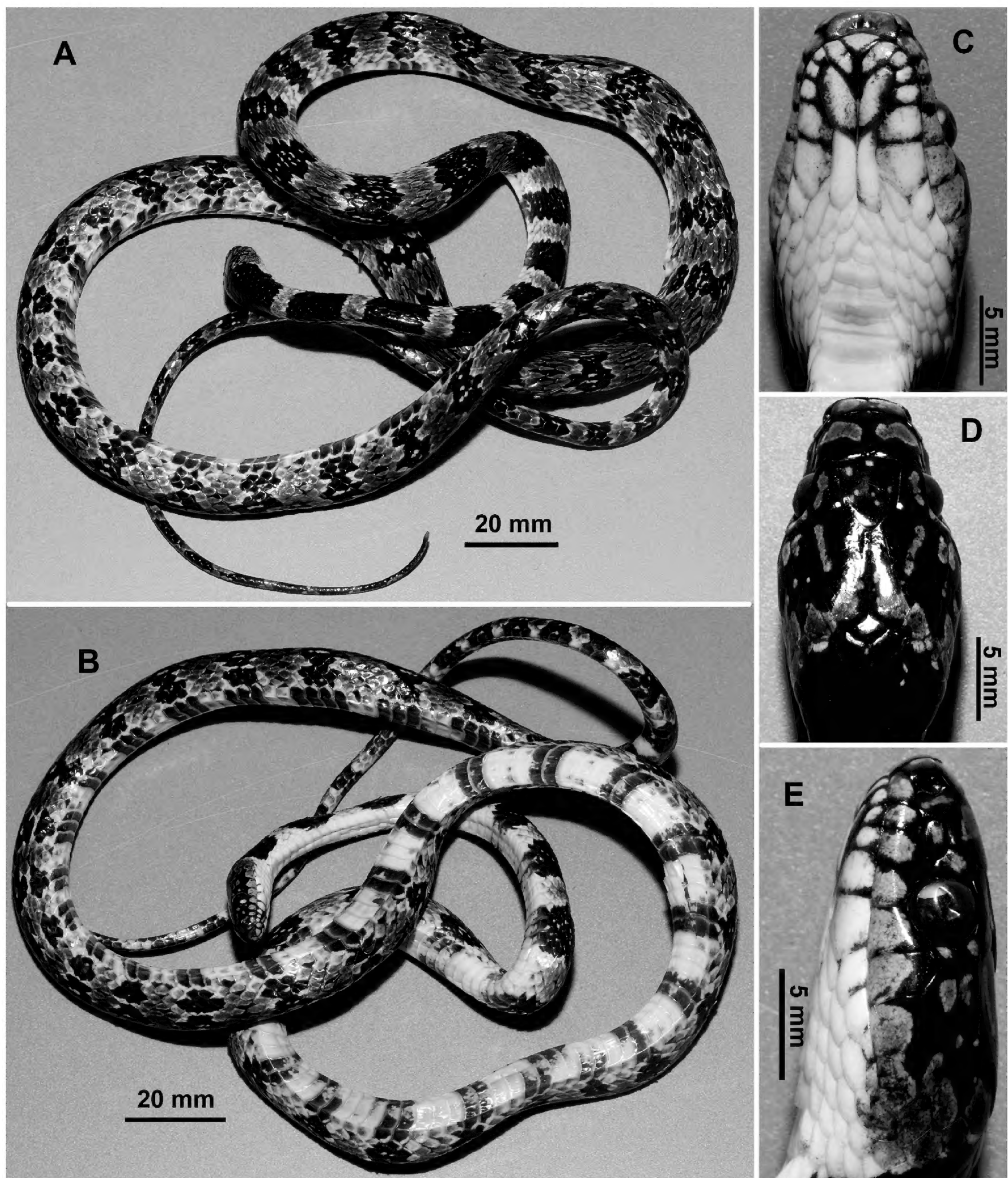
pseudogenes was unlikely (Zhang and Hewitt 1996). The best-fit evolutionary models of the data were: GTR+I+G for the first codon position, HKY+I+G for the second codon position, and GTR+G for the third codon position.

The mtDNA-based BI and ML analyses depicted relatively consistent topologies, with slight disagreement in several shallow nodes (Fig. 4). Both analyses indicated that all putative species of *Lycodon* formed a highly supported lineage (100 PP and 84% UFB). The newly collected specimen formed a clade with *L. fasciatus*, *L. gongshan* Vogel & Luo, 2011, *L. butleri* Boulenger, 1900, and *L. cavernicolus* Grismer, Quah, Anuar, Muin, Wood & Nor, 2014 with high support (100 PP and 97% UFB). Nevertheless, it occupied a basal position in relation to this clade and did not exhibit monophyly with any individual member. Uncorrected *p*-distances among the species within this clade ranged from 7.2% (*L. gongshan* and *L. fasciatus*) to 12.9% (*L. gammiei* and *L. cavernicolus*), while genetic distances between *L. gammiei* and its congeners within this clade ranged from 10.2% to 12.9% (data not shown).

## Discussion

*Lycodon gammiei* is an exceedingly rare species, with a global record of only approximately 10 specimens. The majority of these are from Sikkim and West Bengal, India (Mistry et al. 2007; Chettri and Bhupathy 2009), with only two specimens reported in Cona County, Xizang, China (originally recorded in Eagle-nest Wildlife Sanctuary, India) (Mistry et al. 2007) and Bhutan (Wangyal 2013), respectively. Based on the record by Mistry et al. (2007), Luo et al. (2010) recognized the existence of this species in China, although this recognition has been overlooked in subsequent publications (Wallach et al. 2014; Wang et al. 2020; Uetz et al. 2024). The discovery of this species in Medog County, Xizang, China,

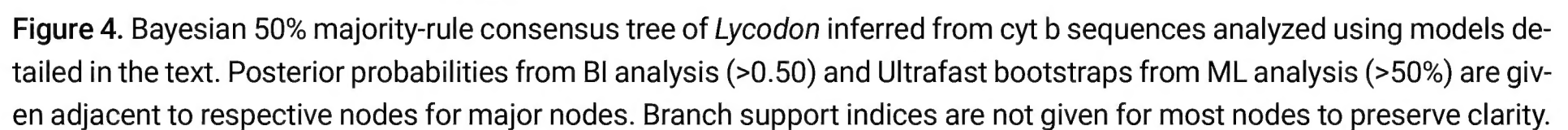




**Figure 3.** Views of the studied specimen (YBU 230088) in preservation. General dorsal (A) and ventral (B) views of specimen, dorsal (C), ventral (D) and lateral (E) views of head (by P Guo).

not only confirms its presence in China but also indicates a further eastward extension of its distribution.

Morphologically, the newly collected specimen shares most characters with the other conspecific specimens (Blanford 1878; Mistry et al. 2007; Chettri and Bhupathy 2009), including eight supralabials (3<sup>rd</sup> to 5<sup>th</sup> touching eye, 6<sup>th</sup> largest), single loreal, 2+3 temporals, one preocular, two postoculars, two genial pairs, cloacal plate entire, and dorsal scales in 17-17-15 rows. However, the new specimen has a greater number of ventral scales (228+1) than all previously reported specimens (205–220) (Mistry et al. 2007; Chettri and Bhupathy 2009).



*Lycodon zayuensis* Jiang, Wang, Jin & Che, 2020 coexists with *L. gammiei* in southeastern Xizang, China (Che et al. 2020; Lyu et al. 2022). Both species exhibit similarities in external morphology, including dorsal scales in 17-17-15



rows, eight supralabials, one preocular, and two postoculars. However, the two species are genetically divergent (Fig. 2), and *L. gammiei* can be easily distinguished from *L. zayuensis* by its broader and fewer yellow body cross-bands (30–43 vs 88–93) (Blanford 1878; Lyu et al. 2022).

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Conceptualization: XM. Formal analysis: BL. Methodology: TZ. Resources: KG. Software: YW. Supervision: PG. Validation: LL. Writing – original draft: FS.

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### Data availability

All of the data that support the findings of this study are available in the main text.

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